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David Bohlender
John Brown University

Stuart Williams
John Brown University

Emir Cruz
John Brown University

H. Gaston Griggs
John Brown University

PHOTOREACTIVATION OF LETHAL DAMAGE INDUCED IN HAMSTER X *XENOPUS* HYBRID CELLS AND THEIR PARENTALS BY UV LIGHT

DAVID BOHLENDER, STUART WILLIAMS, EMIR CRUZ, and GASTON GRIGGS
Department of Biology
John Brown University
Siloam Springs, AR 72761

ABSTRACT

A85 *Xenopus* cells that exhibited a high level of photoreactivation (PR) and V79B2 hamster cells that exhibited little PR were fused to produce the V79B2 × A85 cell line — a hybrid line which possessed a relatively stable karyotype, with most cells containing the entire V79B2 and A85 genomes. UV and UV plus PR fluence-survival relations were then determined and compared for the hybrid and parental lines in a first attempt to elucidate interactions of the parental PR mechanisms in the hybrid. It was anticipated that the A85 genome in the hybrid would produce PR enzyme in sufficient concentration and of such a nature as to efficiently PR UV-induced lethal damage in both A85 and V79B2 DNA, and little difference would be observed in the levels of PR exhibited by the V79B2 × A85 and A85 lines. To the contrary, the level of PR observed for the hybrid was substantially below that observed for the A85 line. To assist in the interpretation of this unexpected observation, three additional preliminary studies were carried out: 1) Comparison of the optimum PR schemes for the A85 and hybrid lines, 2) examination of relations between the PR and dark UV repair mechanisms possessed by these lines, and 3) comparison of the levels of PR of chromatid deletions induced by UV in selected V79B2 and A85 chromosomes of the hybrid. The results suggested that the relatively low level of PR manifested by the hybrid cells was a consequence of their inability to efficiently PR pyrimidine dimers induced by UV in V79B2 DNA.

INTRODUCTION

Previous studies have indicated that A84 amphibian *(Xenopus)* cells possess photoreactivating enzymes and are capable of photoreactivating a relatively high level of lethal damage induced by low doses of UV light (Regan *et al.*, 1968; Griggs and Bender, 1972; Griggs and Orr, 1979; Griggs and Payne, 1981). Photoreactivation (PR) of UV-induced lethal damage has not been clearly demonstrated in V79B1 mammalian (hamster) cells, although photoreactivating enzyme activity has been detected in mammalian cells (Sutherland *et al.*, 1974; Sutherland *et al.*, 1976). Recently, Kulp *et al.* (1985) tried to elucidate the differences in these PR mechanisms by studying interactions of the mechanisms in a V79B1 × A84 hybrid cell line: an approach suggested by the success of previous attempts (*e.g.*, Limbosh, 1982) to describe relations between the radiation repair mechanisms possessed by different parental lines through examining interactions of the mechanisms in hybrid lines formed from the parentals. It was anticipated that the A84 genome in the hybrid would produce PR enzyme in sufficient concentration and of such a nature as to efficiently photoreactivate UV-induced lethal damage (pyrimidine dimers) in both V79B1 and A84 DNA, and the level of PR observed for the hybrid would closely approach that observed for the A84 parental line. To the contrary, the level of PR exhibited by the hybrid did not closely approach that of the A84 line. However, the results of this study were complex and difficult to interpret, possibly due to the fact that the karyotype of the hybrid was not completely stable throughout the experimentation. Thus, we decided to carry out similar experiments with recently cloned hamster and *Xenopus* parental lines (V79B2 hamster and A85 *Xenopus*) and a V79B2 × A85 hybrid line, which possessed a more stable karyotype than the V79B1 × A84 line.

MATERIALS AND METHODS

The A85 *Xenopus* line was cloned from the A8W243 line described by Griggs and Bender, (1972). The V79B2 hamster line was cloned from the B79B1 line described by Kulp *et al.* (1985). The V79B2 × A85 hybrid line was formed by a polyethylene glycol technique similar to that described by Davidson and Gerald (1976). Monolayers for routine maintenance of the cell lines were grown in F12 medium (Gibco) supplemented with 15 percent foetal calf serum (Hazelton) at 31 °C (A85) and 33 °C (V79B2 and V79B2 × A85) in light-tight incubators containing a humidified atmosphere of 5 percent CO_2 in filtered air. Techniques employed in UV irradiations, photoreactivations, caffeine treatments, single cell plating, colony assays, cell synchronizations, mitotic arrest, preparation of chromosome spreads, and chromatid aberration analysis closely paralleled those described by Griggs and Bender (1972), Griggs and Orr (1979), and Kulp *et al.* (1985).

RESULTS AND DISCUSSION

Fig. 1 displays UV and UV + PR fluence-survival relations for A85 and V79B2 parental lines and the V79B2 × A85 hybrid line. The A85 line was substantially less resistant than the V79B2 line under UV treatment; however, only the *Xenopus* line exhibited a detectable level of photoreactivation. The V79B2 × A85 hybrid line was somewhat more resistant than either parental line to UV irradiation, yet seemed to lack the efficient PR repair capability of the A85 line. These results were quite similar to those obtained by Kulp *et al.* (1985) with their unstable V79B1 × A84 hybrid line, and suggested that the relatively low photoreactivation repair potential of that hybrid line was not due entirely to karyotype instability. These alternative explanations were then examined:

1. Perhaps the PR mechanisms for the A85 line and the V79B2 × A85 line differ in such a manner as to require that significantly different schemes for administration of photoreactivating light be employed with each line to produce optimal PR for that line. The photoreactivating light administration scheme employed in establishing the UV + PR curves for Figure 1 may have significantly favored the A85 line and a higher level of PR might have been observed in the hybrid line if an

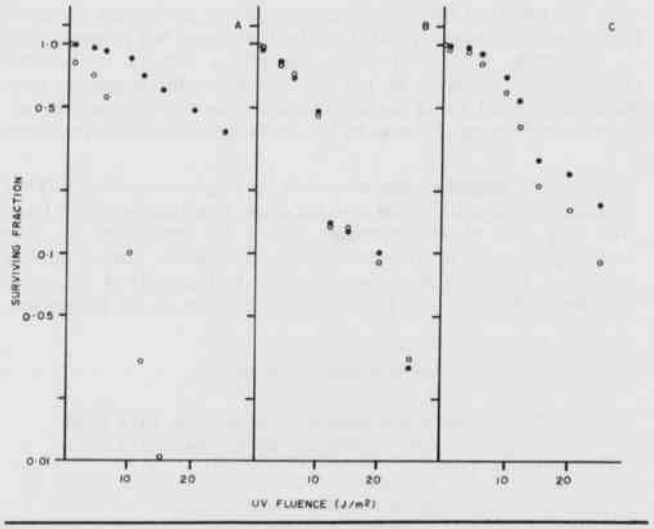

Figure 1. The UV (open circles) and UV + PR (filled circles) survival curves for A85 cells (A), V79B2 cells (B), and V79B2 × A85 hybrid cells (C). Photoreactivating light was administered at 24 °C. The fluence rate and total fluence were 10w/m² and 25 × 10³ J/m² respectively. All irradiations were carried out under red light.

optimal scheme for it had been employed. To test this explanation, experiments were conducted to compare the optimal PR light administration schemes for the cell lines. These schemes were considered to be composed of optimal values for three parameters: temperature, fluence rate, and total fluence. The results indicated that optimal value "ranges" existed for the three parameters and the range for each parameter was the same for both cell lines (temperature, 20-24 °C; fluence rate, 5.0-15.0 w/m²; total fluence, 20,000-28,000 j/m²). Furthermore, the parametric values used in the scheme for establishing the UV + PR curves for the two cell lines fell within these ranges (caption for Fig. 1). Thus, suggested explanation (1) appears contradicted.

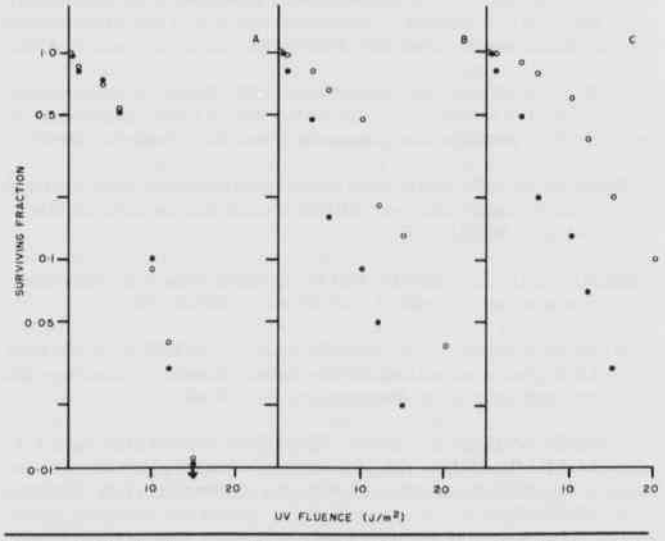

Figure 2. Survival curves following UV (open circles) and UV + CF (filled circles) for A85 cells (A), V79B2 cells (B), and V79B2 × A85 cells (C). The cells were incubated in a medium containing 0.80mM/l caffeine from immediately following irradiations until termination of experiments.

2. The data of Figure 1 coupled with that of Figure 2 suggest that the hybrid line and the A85 parental line may possess similar PR mechanisms, but only the hybrid line possesses an efficient caffeine sensitive repair CSR mechanism. In a caffeine (CF) free hybrid intracellular environment, the CSR mechanism successfully competes with the PR mechanism for UV-induced pyrimidine dimers in the hybrid DNA and this competition reduces PR to the relatively low level observed. In a hybrid intracellular environment containing CF, CSR should be blocked, CSR competition eliminated and a higher level of PR observed. Efficient CSR does not exist in the A85 intracellular environment and, thus, a much higher level of PR was observed for the A85 line. Specifically, explanation (2) proposes that if samples 1 and 2 of the hybrid cells were treated with 10J/m² UV + 0.80 mM/1 CF + 25 × 10³ J/m² PR and 10J/m² UV + 0.80 mM/1 CF respectively, while samples 3 and 4 were treated with 10J/m² UV + 25 × 10³ J/m² PR and 10J/m² UV respectively, then one would expect SF1 − SF2 > SF3 − SF4 (where SF1 = surviving fraction of sample 1, etc.) Samples of cells were treated in this fashion to test the inference and the results are recorded in Table 1. Comparison of the normalized surviving fractions of experiments 1 and 2 with those of experiments 3 and 4 indicates that SF1-SF2 > SF3-SF4 and explanation (2) is contradicted.

Table 1. Survival of V79B2 × A85 cells following UV, UV + CF, UV + PR, and UV + CF + PR treatments.

Experiment number *	UV flugnce (J/m²)	PR flugnce (J/m²)	caffeine concentration (mM/liter)	Number of cells isolated	Normalized surviving fraction
0	0	0	0	6,000	1.00
1	10	25,000	0.80	6,000	0.26
2	10	0	0.80	6,000	0.16
3	10	25,000	0	6,000	0.68
4	10	0	0	6,000	0.56

* Cell sample number

3. A low UV fluence would be expected to induce in V79B2 and A85 chromosome segments of equal length approximately the same number of pyrimidine dimers that lead to a given end point. However, perhaps the A85 PR mechanism photoreactivates the dimers in A85 chromosomes more efficiently than it does the dimers in V79B2 chromosomes. Thus, since the hybrid cells contained both A85 and V79B2 chromosomes, while the A85 cells contained only A85 chromosomes, the A85 cells were capable of photoreactivating a greater fraction of their UV-induced dimers leading to the end point cell death than were hybrid cells. Since chromosomal aberrations are also endpoints for pyrimidine dimers (Griggs and Bender, 1973), this proposed explanation predicts that, following a given UV fluence, the hybrid cells should also photoreactivate aberrations induced in A85 chromosomes more efficiently than aberrations induced in V79B2 chromosomes. The experiment described next was performed to test this prediction.

Six sets of synchronous G1 phase hybrid cells were prepared. Set 1 received no radiation; set 2 received 25 × 10³ J/m² PR light; set 3 received 14.0 J/m² UV; set 4 received 14.0 J/m² UV + 25 × 10³ J/m² PR light. Each set was then separated into two subsets, a and b. Subset a cells were allowed to progress to mitosis. Samples of subset b cells were used for mitotic index monitoring to determine the appropriate time for colcemid collection of subset a mitotic cells for preparation of metaphase chromosomes spreads. These spreads contained a number of small V79B2 and A85 metacentric chromosomes very similar in appearance and so difficult to distinguish that a detailed aberrational analysis of the entire karyotype did not appear feasible. Instead, the aberrational analysis was restricted to comparing the number of chromatid terminal deletions produced by the various treatments in an

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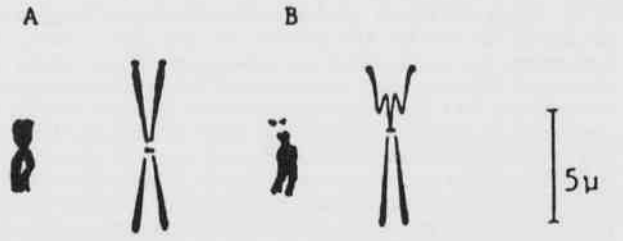

Figure 3. Photographs and schematics for (A) the V79B2 chromosome and (B) the A85 chromosome used in the PR study of UV-induced chromatid deletions.

A85 chromosome and a V79B2 chromosome which were approximately equal in length and easily distinguished. The V79B2 chromosome was a metacentric with small satellites (Figure 3A) and the A85 chromosome was a submetacentric with what appeared to be aberrant supercoiling in one arm (Fig. 3B). The resulting data, displayed in Table

Table 2. PR of chromatid deletions induced by UV in two chromosomes of G1 phase V79B2 × A85 cells.

Experiment number *	UV fluence (J/m^2)	PR fluence (J/m^2)	Cell Collection time range (h after UV)**	Number cells scored	Mean number of deletions/trail ± standard errors observed in the chromosomes A85	V79B2
1	0	0	22-30	1200	2	3
2	0	25,000	22-30	1200	2	4
3	14	0	42-56	1200	36.0 ± 0.6	39.3 ± 0.81
4	14	25,000	22-34	1200	7.0 ± 1.0	31.4 ± 1.50
5	18	0	50-65	1200	45 ± 1.8	44.6 ± 1.3
6	18	25,000	22-40	1200	10 ± 2.1	33.0 ± 2.2

* Each experiment consisted of three trials with 400 cells scored in each trial.
** Metaphase spreads were collected by colchemid treatments that spanned the indicated time ranges.

2, reveals that the difference in the numbers of deletions observed for the two chromosomes following a given UV exposure is rather small compared to the difference in the numbers of deletions observed for the two chromosomes following that UV exposure plus PR. For example, comparison of the chromatid data of experiments 3 and 4 for the A85 chromosome indicates that PR reduces the number of deletions induced by 14.0 J/m^2 UV from 36.0 ± 0.6 to 7.0 ± 1.0 (approximately 80 percent of the deletions photoreactivated), while comparison of the data of these same experiments for the V79B2 chromosome indicates that PR reduces the number of UV-induced deletions from 39.3 ± .081 to 31.4 ± 1.50 (only about 20 percent of the deletions photoreactivated). These data tend to confirm the prediction of proposed explanation 3 and, thus, support this proposal.

In conclusion, the results of the UV and UV + PR fluence-survival experiments tend to confirm the earlier, somewhat unexpected, observation by Kulp *et al.* (1985) that hamster X *Xenopus* hybrid cells do not PR UV-induced lethal damage as efficiently as *Xenopus* parental cells. The results of additional experimentation, performed to explain this observation, suggest that the hybrid cells may not possess ability to efficiently PR UV-induced pyrimidine dimers in hamster chromosomes. However, the key data supporting this suggestion is indirect and limited, being results of an experiment to compare the percentages of deletions, induced by UV, in a single hamster chromosome and a single *Xenopus* chromosome that can be photoreactivated. Thus, questions appropriately arise as to the extent to which the results found with this pair of chromosomes should be generalized to other heterologous pairs of hybrid chromosomes. Similar aberrational experiments, involving larger segments (or perhaps all) of the hybrid genome would constitute a more convincing test of the suggestion.

ACKNOWLEDGEMENT

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